SEED CHARACTERISTICS IN SOYBEAN

BY

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by

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In memory of my father Valdomiro A. Almeida.

Dedicated to my mother, Maria C. Jesus,

my son Estêvão Mascaro de Almeida, and

my daughter Clarissa Mascaro de Almeida.

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Poor quality of soybean seed has been often associated with seedborne fungi. The effects of date of planting and maturity, and harvest time on the incidence of seedborne fungi were studied in experiments conducted in 1986 and 1987. In each year, five genotypes were grown on six different planting dates at Gainesville, FL. Seeds were harvested at maturity (H_0), one week later (H_1), and two weeks later (H_2). The blotter test was used to assess fungal infection and seed germination. Results from the two years were different primarily due to more moisture during the period of seed maturation in 1986 and a relatively dry season in 1987. Extensive fungal infection was achieved only under high moisture conditions. Phomopsis spp., Fusarium spp., and Colletotrichum truncatum, in that order, were the most important fungi that infected seeds. The

incidence of other fungal species that caused seed deterioration was insignificant. Extensive infection of seed by C. truncatum occurred only in early-maturing seeds subjected to high temperatures and heavy rainfall. Extensive infection of seeds by Phomopsis sp. and Fusarium sp. occurred when seeds matured under conditions of high humidity. Early planted and early maturing genotypes had the highest percentage of seed with fungal infection and the lowest percentage of seed germination. Fungal infection of seed increased substantially with delay in harvest and seed germination declined proportionally to the infection level.

The inheritance of resistance of seeds to Phomopsis infection was studied in crosses between PI 417479 (resistant) and susceptible cultivars. In F_2 populations, two dominant genes were involved in the resistance of seed to decay by Phomopsis. Evidence that minor genes influenced resistance was observed. Broad-sense heritability estimates ranged from 0.33 to 0.71, thus effective selection to resistance can be attained by using appropriate breeding procedures. The incidence of seed infected by Phomopsis and by all fungal species were highly correlated with percentage of seed germination in blotter test and ranged from -0.70 to -0.99. Selection based on seed germination in blotter tests would improve resistance in soybean to general pathological and physiological causes of seed deterioration.

The inheritance of a new phenotype with a brown pigmentation confined to the seedcoat in the radicle-hypocotyl axis region (brown radicle) was studied in five crosses. In the classification of seeds from F_2 plants, the brown radicle was determined to be inherited as a single gene. Gene symbol **rd** is proposed. Expression of **rd** may be influenced by modifying genes. A close linkage between the **rd** and **I** (light hilum) loci was indicated. Complete independence was observed between **the rd** and pubescence color **T** loci.

CHAPTER I
INTRODUCTION

Soybean [Glycine max (L.) Merrill] is one of the most important crops in the world and is grown for its high content of quality protein and excellent edible oil. The crop is more traditionally grown in temperate latitudes. In the last decade, however, there has been a great expansion of production in tropical areas. A major constraint to the soybean production in many areas of the world, particularly tropical and subtropical areas, is related to the production and maintenance of high quality seed.

Under conditions of high temperature and high humidity, soybean seeds deteriorate rapidly (Alexander et al., 1978; Wilcox et al., 1974). The deterioration, in general, is caused by physiological and pathological factors. The deterioration process in the field occurs after physiological maturity (Teckrony et al., 1987). Soybean seed with poor quality has often been associated with seedborne pathogens. Phomopsis spp. are believed to be the most important fungi, although in some production areas other fungal species can also occur in high incidence (Mc Gee, 1986).

Physiological factors to improve seed quality in soybean are not yet well identified. Thus, breeders are currently concentrating on resistance to fungal infection of seed as an adjunct to improve seed quality. Resistance to Phomopsis decay is expected to improve greatly seed quality. Also, the knowledge of the mechanism of resistance and a better understanding of this disease would be important to implement breeding programs for improved seed quality.

One series of experiments was designed to study characteristics in soybean seed related to fungal infection. In another series of experiments, the inheritance of a new phenotype for seedcoat pigmentation was studied.

The objectives reported in Chapter II were to determine i) the extent to which maturity and harvest time influenced seed quality, ii) which pathogens were present in seeds maturing at different times, iii) if the relative importance of each pathogen changed during the maturation period, and iv) which maturity dates were more suitable for effective selection for resistance to seed infection.

The main objective of the study reported in Chapter III was to determine the genetic basis of resistance to infection of seeds by Phomopsis in the genotype PI 417479. Also a exploratory genetic study was carried out to obtain more information on the resistance of F85-4901, a breeding line with good resistance to the pod and stem blight disease caused by the Phomopsis / Diaporthe complex.

The objective of the study reported in Chapter IV was to determine the inheritance of brown radicle, a pigmented pattern confined to the radicle-hypocotyl area in the soybean seedcoat.

CHAPTER II
EFFECT OF GENOTYPE, PLANTING AND MATURITY DATE, AND HARVEST DELAY ON THE INCIDENCE OF SEEDBORNE FUNGI IN SOYBEAN

Deterioration of soybean seed in the field is associated with unfavorable environmental conditions during the seed-ripening process and conditions during the period from seed maturity to harvest. The most important factors that cause poor quality seed are temperature and moisture (Mandagron and Potts, 1974; Tekrony et al., 1980; Wilcox et al., 1974). The deterioration is more pronounced in tropical climates where high temperature and humidity normally occur during this period (Dassou and Kueneman, 1984; Paschal II and Ellis, 1978).

Fungal pathogens on seed are almost always associated with field weathering (seed deterioration). Phomopsis spp. are believed to be the major contributors to reduce quality of soybean seed when ripening occurs under conditions favoring development of the fungi (Kulik and Schoen, 1981; Tekrony et al., 1983). However, the interaction between pathological and physiological causes is not well determined. A synergistic effect is expected to occur between infection by Phomopsis spp. and field weathering,

since both factors are affected by the same environmental conditions. Based on experiments carried out under tropical condition, Ndimande et al. (1981) reported that pathogens seem to be important agents in seed deterioration prior to harvest.

The effect of moisture and temperature on infection of soybean seed by Phomopsis spp. is well documented. Shortt et al. (1981) reported that rainfall during the pod-filling period was more closely associated with the occurrence of Phomopsis seed decay than was temperature. Tekrony et al. (1983) observed that high incidence of Phomopsis spp. was significantly correlated with air temperature and minimum relative humidity during seed development, but not with precipitation. Based on their prediction model they concluded that infection of soybean seed depended more on moisture than on temperature. However, Wilcox et al. (1974) reported that warm and wet condition during seed development and maturation favored fungal infection of seeds.

Infection of pods by Phomopsis spp. takes place from flowering onward. However, extensive infection of seeds is more likely to occur during and after the physiological maturity (stage R_7 described by Fehr and Caviness, 1977). Balduchii and McGee (1987) examined the effect of moisture and temperature on infection of seeds during the maturation process (R_7 to R_8). They found that at least three continuous days at high humidity (100%) were needed to

achieve extensive infection at 25 ^{0}C. However, it took four and five continuous days to attain the same level of infection when temperature was reduced to 20 and 15 °C, respectively. The relationship of moisture of pods and seeds to the rate of infection of seeds by Phomopsis spp. was also studied by Rupe and Ferriss (1986). They concluded that this fungus has the ability to grow and infect soybean seed whenever environmental conditions maintain pods at a water content above 19%. This is in close agreement with the generalized opinion that high moisture after physiological maturity, which retards pod drying, is conducive to increased fungal infection and seed deterioration (McGee, 1986; Thomison et al., 1987). Moreover, Vaughan et al. (1989) also reported that the reproductive growth period from R6 to R7 was more critical to Phomopsis infection of seed than the period following R7 (physiological maturity).

Other pathogens can also be important problems in seed production, depending on the geographical region where the crop is grown. Several authors have reported high incidence of seed infected by Colletotrichum truncatum (Schw.) Andrus and Moore and Fusarium spp. in soybean seed produced in tropical and subtropical regions. Levels above 50% of seed infected by Colletotrichum have been reported in seed lots produced in Brazil (Henning, 1988). Nicholson and Sinclair (1973) reported that C. truncatum and Fusarium spp. were

among the fungi most frequently isolated in India. These fungi were also considered important pathogens in seed deterioration in Nigeria (Ndimande et al. 1981). Phomopsis spp. and Fusarium spp. were the fungi most frequently associated with soybean seed produced in Northwest Florida in 1986, yet in some seed lots, C. truncatum occurred in a relatively high incidence (Franca Neto, 1989). An antagonistic effect of C. truncatum on infection of seed by Phomopsis was reported by Hepperly et al. (1980) and Indra et al. (1985). Krare and Chacko (1983) reported that seed infection by Colletotrichum was higher in early plantings in India because maturation coincided with heavy rain and high temperatures. Also, a dense canopy resulted in higher incidence of infected seed.

Weeds have been associated with low seed quality in soybean. The weeds can act as alternate hosts or provide a microclimate of prolonged high humidity that favors a high incidence of seeds infected by Colletotrichum, Fusarium, and Phomopsis (Dhingra and da Silva, 1978).

The effects of planting and maturing date and delayed harvest on the incidence of seedborne pathogens have been reported in several papers. Fungal infection of seeds has been shown to be higher in early-maturing cultivars compared to late maturing ones. Similarly, infection is higher in early-planted than in late-planted soybeans (Nicholson and Sinclair, 1973; Tekrony et al., 1983; Wilcox et al., 1974,

1985). These differences in infection among cultivars were related to the environmental conditions during seed development and maturation (Alexander et al., 1978). Wilcox et al. (1985) concluded that the superior seed quality of the late-maturing genotypes was not caused by genetic resistance, but by escape from infection. When forced to mature at the same time, the incidence of seed infected by Phomopsis spp. in the late maturity genotypes increased to the same or higher levels as those observed in early maturity group. High temperature and moisture during the early maturation were the causes of increased infection of seeds (Wilcox et al., 1974).

A similar explanation has been suggested for increased infection and poor seed quality associated with delayed harvest (Ndimande et al., 1981; Paschall II and Ellis, 1978; Wilcox et al., 1974). Alexander et al. (1978) found that unharvested seed deteriorated rapidly after reaching maturity under Florida conditions. Fungal infection resulted in lowered seed germination.

Delayed harvest after maturity has been used frequently as a screening technique to (i) identify cultivars and breeding lines with characteristics of good seed quality (Dassou and Kueneman, 1984; Paschall II and Ellis, 1978), (ii) assess reactions to diseases causing seed deterioration (Alexander et al., 1978; Wilcox et al., 1985), and (iii) improve both the physiological and pathological resistance

to field weathering (Ndimande et al., 1981). Dassou and Kueneman (1984) pointed out that this technique has some limitations because environmental effects could confound comparisons among genotypes of different maturity. This was later corroborated by Wilcox et al. (1985) who found that this technique was not effective in years with low incidence of fungal infection. Under tropical conditions, marked variation in temperature is unusual during the growing season, and the humidity may remain relatively high during and after seed maturation (Paschall II and Ellis, 1978). In Florida, the period of least rainfall during the growing season was found to occur during the last two weeks of October and the first three weeks of November (Alexander et al., 1978); a typical tropical climate occurs during the growing season and before mid-October when daily temperatures begin to drop.

The incidence and prevalence of different fungal species in seeds maturing at different times has not been well studied in Florida. Therefore, it is not known if the relative importance of each pathogen changes with maturity date and delay in harvest. The objective of this study was to: (i) determine the extent to which maturity date and delayed harvest influenced seed quality, (ii) determine which seed pathogens were present in seeds maturing at different times, (iii) determine if the relative importance of each pathogen changed with maturity dates, and

(iv) determine which maturity dates were most suitable for effective selection for resistance to Phomopsis and other fungi that infect seed.

Materials and Methods

The experiments conducted in this study were part of ongoing research to study the response of long juvenile soybean genotypes to planting date.

Among various isoline pairs of long juvenile and conventional types planted in these experiments, a group of five genotypes were selected to be grown in six experiments on planting dates in 1986 and 1987. This group was composed of two conventional cultivars—'Forrest' (group V), and 'Kirby' (group VIII), and three breeding lines— F85-994 (group III), F85-998 (group V), and F85-1108 (group V).

The experiments were conducted at the Agronomy Farm, University of Florida, Gainesville, Florida, on an Arredondo fine sand, a loamy, siliceous, Grossarenic, Paleudult soil. The experimental design was a randomized complete block in a split plot arrangement, with two replicates per treatment. Whole-plot treatments were the five cultivars. Sub-plot treatments consisted of three time-spaced harvests described as (i) H_o- promptly harvested at harvest-maturity stage (R_8), (ii) H_1- harvested 7 days after the R_8 stage, and (iii) H_2- harvested 14 days after R_8. Each field plot

consisted of a single row 210 cm in length with a 91 cm spacing between rows. Seeding rate was 33 seeds per meter of row. The six experiments established in 1986 were sown on 6 May, 21 May, 17 June, 1 July, 15 July, and 24 July.

Pre-emergence herbicide alachlor (2-chloro-2',6'-diethyl N-(methoxymethyl)-acetanilidine) was used for weed control, complemented with hoe weeding when needed. Fertilizer was applied according to needs indicated by soil analysis, and sprinkler irrigation was applied as needed during the growing season. The experiments were frequently scouted for insect pests. Control measures were taken before levels causing economic damage were reached.

Plots were examined for uniformity in maturation at the end of R_7 stage when plants had a few dried-out brownish pods. Only those plants at the same maturation stage were kept; plants with advanced or delayed maturation, as well as two to four plants at both row extremities were trimmed. At harvest maturity (H_0), three to five random plants were sampled in each experimental plot, identified, dried if needed, and stored at room temperature for subsequent threshing. The same procedure was adopted in those harvest performed 7 (H_1) and 14 days later (H_2).

The sample-plants were threshed in an individual plant thresher and the seeds stored in a cold room (17 ^{0}C; 65% RH)till pathologic tests were performed one to two months later.

The blotter test was used to determine the incidence of fungal infection. A sample of 20 random seeds was taken from each sample-plants and placed in Petri dishes (14 x 2.5 cm) lined with two layers of moist germination paper. Seeds with apparent mechanical or stink-bug damage were discarded. Petri plates containing the seeds were incubated for 7 days at 25 C and under a regime of 8 hours fluorescent daylight. The fungal incidence was recorded by visual examination of the colonies grown from infected seeds. The following fungi were identified: Phomopsis spp., Colletotrichum truncatum (Schw.) Andrus and Moore, and Fusarium spp. Total incidence of fungal infection was obtained by summing the percentage of seeds infected with the three genera. Individual seed infected by two fungal genera were counted twice for the total-infection category. Additionally, germination percentage was determined based on the number of seedlings produced in the blotter test.

The six experiments established in 1987 were sown in 30 April, 3 June, 11 June, 30 June, 17 July, and 24 July. The materials and methods were similar to those described for 1986.

The daily maximum and minimum air temperature, and precipitation were obtained from IFAS climatological monitoring station, located about 500 meters from the sites where the experiments were conducted in both years.

Data for all variables were converted to arcsine (percentage)$^{1/2}$ prior to the appropriate analyses of variance. Maturity dates were converted to days after 1 Aug. MSTAT, a PC statistical program, was used for data analyses. The combined analyses over years, and planting dates followed those procedures described by McIntosh (1983). Years and planting dates were considered random effects, while genotypes and harvest times were fixed effects. Treatment means were tested for significance using the LSD tests at the 5% level of probability.

Results and Discussion

In the combined analyses of variance, highly significant differences were found between years for all variables except for infection by Colletotrichum (7.6 vs. 7.2%). The much higher percentage of infection in 1986 for Phomopsis (36.2 vs. 10.3%), Fusarium (16.9 vs. 4.2%), and total infection (60.7 vs. 21.7%) apparently was associated with higher rainfall in 1986 from mid-August through October (Fig.2.1) when developing and mature seeds were exposed. For this reason, data for the two years will be discussed separately.

1986 Results

Significant differences among nearly all main effects in 1986 and some significant interactions among factors were

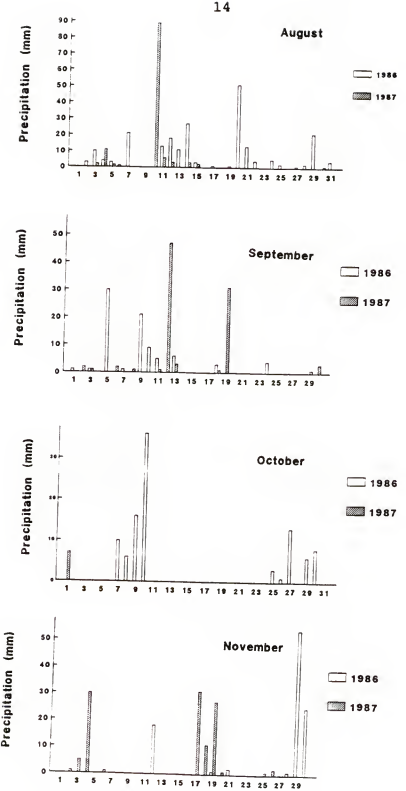

Figure 2.1. Precipitation from August to November in 1986 and 1987, at Gainesville, FL.

found (Table 2.1). Planting date significantly influenced percentage of infection by Phomopsis. The highest rate of infection (47.7%) was in seeds from the first planting date (6 May) and there was a consistent decline in incidence to 23.3 % for date 5, planted 15 July, and a slight but non-significant increase for date 6 (Table 2.2).

Several factors probably contributed to the higher incidence of Phomopsis in the earlier planted plots. They matured earlier in the fall (Table 2.3). In temperate regions early maturity was almost always associated with more Phomopsis infection (Nicholson and Sinclair, 1973; Wilcox et al., 1974). They had longer life cycles (Table 2.3) which exposed them to inoculum and infection for longer periods. The longer life cycle also resulted in more vegetative growth which reduced air movement and sunlight penetration for drying. Because mechanical cultivation ceased relatively early in the earlier planted plots, late season weeds were more prevalent which further contributed to higher humidity in and around plots.

Genotypes differed in percentage of infection by Phomopsis (Table 2.1). The range was from 46.7% for Forrest to 23.2% for F85-994 (Table 2.2). Some of the apparent resistance of F85-994, however, may have resulted from antagonism from Colletotrichum, as described by Hepperly et al. (1980).

Table 2.1. Mean squares and F-tests on the incidence of fungal infection of seed and seed germination computed for experiments combined over planting dates in 1986.

Source	d.f.	*Phomopsis* spp.	*C. truncatum*	*Fusarium* spp.	Total infection	Germination
Planting date (P)	5	2724.8**	1637.2**	392.4 ns	13538.2**	5560.6**
Genotype (G)	4	2628.2*	6940.1**	4075.4**	11060.6*	7762.6**
P x G	20	873.5**	613.6**	521.8**	2628.4**	1268.9**
Harvest time	2	14397.4**	591.4**	6691.6**	75729.9**	37943.0**
P x H	10	142.7 ns	90.8 ns	137.9 ns	747.9**	273.7ns
G x H	8	222.8 ns	128.0 ns	229.8 ns	1168.3*	491.0ns
P x G x H	40	170.6**	90.8 ns	127.6 ns	512.5**	325.0*
Mean		36.2	7.6	16.9	60.7	44.4
C.V.		28.2	73.2	34.6	19.1	22.6

*, ** indicate significance at 5 and 1%, respectively.

Table 2.2. Observed values and deviations from expected values for percentage Phomopsis infection of seed in 1986.

Planting date	F85-994	Forrest	F85-998	F85-1108	Kirby	Mean
	%					
PD_1	30.0 +0.6	50.0 +11.5	55.8 -3.5	51.7 -0.5	50.8 -8.1	47.7 (43.7)*
PD_2	20.8 +8.8	60.8 -1.2	65.8 -15.1	60.0 -10.4	23.3 +18.0	46.2 (43.0)
PD_3	24.2 -1.3	47.5 -1.4	15.8 -6.6	44.2 -5.8	16.7 +15.3	35.7 (36.7)
PD_4	26.7 -6.2	41.7 -0.4	30.0 +5.1	37.5 -3.1	24.2 +4.4	32.0 (34.4)
PD_5	25.8 -10.9	36.7 -6.6	14.2 +11.4	8.4 +16.6	31.7 -10.8	23.3 (28.9)
PD_6	11.7 +8.9	43.3 -1.8	26.7 +8.6	31.7 +2.9	47.5 -18.7	32.2 (34.6)
Mean	23.2 (28.8)!	46.7 (43.1)	39.7 (39.1)	38.9 (38.6)	32.4 (34.7)	36.2

( ) arcsine $(\%)^{1/2}$ transformed means are presented in parentheses.
* LSD (0.05) = 10.2
! LSD (0.05) = 9.6

Table 2.3. Dates of harvest maturity (R_8) and days to maturity in five soybean cultivars planted at six planting dates in 1986.

	PD_1*	PD_2	PD_3	PD_4	PD_5	PD_6
	5/6	5/21	6/17	7/01	7/15	7/29
F85-994	08/18 104	08/28 99	09/19 94	09/26 87	10/03 80	10/14 77
Forrest	09/08 125	09/18 120	10/03 108	10/03 94	10/17 94	11/03 97
F85-998	09/06 123	09/18 120	10/01 106	10/11 102	10/24 101	11/07 101
F85-1108	08/28 114	09/05 107	09/26 101	10/13 104	10/21 98	11/01 95
Kirby	10/13 160	10/16 148	10/21 126	10/27 118	11/01 109	11/07 101

* PD = planting dates.

The highly significant interaction for planting date x genotype (Table 2.1) is of particular interest for inferences about effects due to maturity date. Observed values for incidence of Phomopsis for each genotype-planting date combination are shown in Table 2.2. To identify the source of the interaction, expected values for each planting date-genotype combination were calculated using the procedure for calculating expected values in the chi-square test for independence. Expected values are shown as deviations from observed. For example, for Forrest in PD_1 the expected value was 61.5% or +11.5% from observed. The larger positive deviations from observed (lower than expected incidence of Phomopsis) generally were for maturity dates between 16 and 24 Oct.

In Table 2.2, however, effects of planting date and maturity date are confounded. Effects of maturity date can be best seen in Table 2.4, prepared from combining data in Table 2.2 and 2.3. Two means for maturity date are shown, with and without F85-994. Values for F85-994 were atypical. The lowest incidence of seed with Phomopsis infection occurred when plants matured between 21 and 27 Oct. (Table 2.4), when temperatures were lowest (Fig.2.2) and rainfall was absent (Fig.2.1) from 11 through 24 Oct. The increased incidence of Phomopsis in seeds that matured in early November probably was caused by the rainy period from 25 to 30 Oct. (Fig.2.1) and the relatively high minimum

Table 2.4. Incidence of Phomopsis infection of seed in five genotypes maturing at different maturity ranges in 1986.

Maturity range	F85-994	Forrest	F85-998	F85-1108	Kirby	Mean
			%			
08/18- 08/28	30.0 20.8			51.7		34.2 (51.7)*
09/05- 09/08		50.0	55.8	60.0		55.3 (55.3)
09/18- 09/19	24.2	60.8	65.8			50.3 (63.3)
09/26- 10/03	26.7 25.8	47.5 41.7	45.8	44.2		38.6 (44.8)
10/11- 10/17	11.7	36.7	30.0	37.5	50.8 23.3	31.7 (35.7)
10/21- 10/27			14.2	8.4	16.7 24.2	15.9 (15.9)
11/01- 11/07		43.3	26.7	31.7	31.7 47.5	36.2 (36.2)

* values in parentheses are means without F85-994

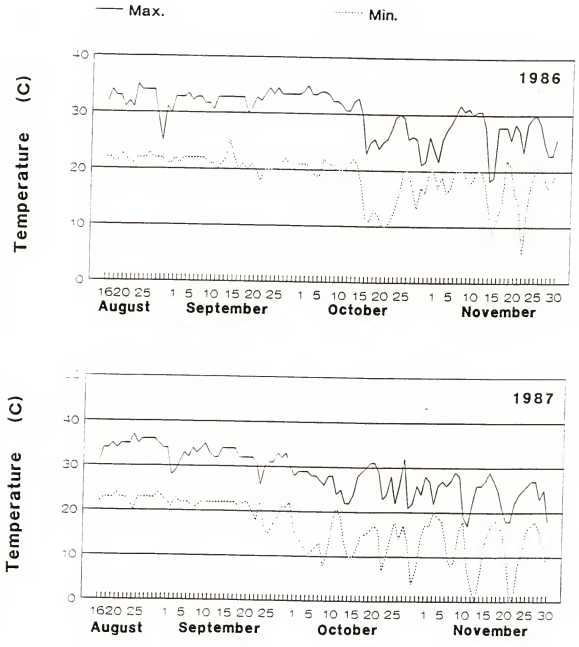

Figure 2.2. Maximum and minimum temperatures from August to November in 1986 and 1987, at Gainesville, FL.

temperature beginning about 24 Oct. (Fig.2.2). The highest incidence was in the two periods extending from 5 to 19 Sept. The frequent rainfall from 20 Aug. through 13 Sept. (Fig.2.1) and generally high temperatures throughout this period (Fig.2.2) apparently were very favorable for Phomopsis infection as was found by Alexander et al. (1978).

Harvest time had the largest and most consistent effect on Phomopsis infection. Seeds harvested at maturity averaged 19.2% infection; those harvested one week later averaged 39.8%, and those harvested two weeks after maturity averaged 49.5% (Table 2.6). The large increase in incidence from H_0 to H_1 would be expected to have a detrimental effect on seed germination. The interactions of harvest time with genotype and planting date were non-significant.

Planting date influenced the incidence of Colletotrichum on seed (Table 2.1). The highest incidence (17.8%) was in seeds from the first planting date and there was a consistent decline in incidence to 3.4% in seeds from planting date 6 (Table 2.5).

There was a very large genotype effect for incidence of seed with Colletotrichum (Table 2.1). The highest level (22.6%) was in F85-994 followed by a lower level in Forrest (10.8%). The other three genotypes were significantly below Forrest and not different from each other (Table 2.6).

The effects of maturity date which probably contributed substantially to the planting date x genotype interaction

Table 2.5. Effect of date of planting and maturity on the incidence of seeds with fungal infection and percentage of seed germination in 1986.

Planting date	C. truncatum	Fusarium spp.	Total infection	germination
	----------		% ----------	
PD_1	17.8(24.9)*	14.7	80.2(63.5)	28.3(32.1)
PD_2	10.2(18.6)	18.0	74.4(59.6)	32.2(34.6)
PD_3	8.7(17.2)	18.7	63.0(52.5)	42.0(40.4)
PD_4	3.0(10.0)	14.3	49.4(44.7)	52.8(46.6)
PD_5	2.5(09.1)	14.5	40.4(39.5)	61.2(51.5)
PD_6	3.4(10.6)	21.3	56.9(49.0)	49.7(44.8)
Mean	7.6	16.9	60.7	44.4
LSD (0.05)	(08.1)		(13.7)	(12.1)

Maturity date	A	B			
08/18 - 08/28	48.8	3.3	14.7	82.5	29.2
09/05 - 09/08	22.5	2.6	22.2	86.7	19.2
09/18 - 09/19	12.5	1.7	18.3	77.5	23.9
09/26 - 10/03	9.2	5.5	14.9	63.1	39.1
10/11 - 10/17	5.0	0.9	11.4	46.3	56.3
10/21 - 10/27	_	0.2	15.4	31.5	59.8
11/01 - 11/07	9.2	0.4	21.3	59.7	48.7

* arcsine $(\%)^{1/2}$ transformed means are presented in parentheses.
A = F85-994 + Forrest B = F85-998 + F85-1108 + Kirby

Table 2.6. Effect of genotype and harvest time on the incidence of seeds with fungal infection and percentage of seed germination in 1986.

Genotype	Phomopsis spp.	C. truncatum	Fusarium spp.	Total infection	germination
	----------	----------	%	----------	----------
F85-994	23.2(29)*	22.6(28)	12.5(21)	58.4(50)	45.6(42)
Forrest	46.7(43)	10.8(19)	13.4(21)	71.0(57)	31.3(34)
F85-998	39.7(39)	3.1(10)	19.9(26)	62.7(52)	36.3(37)
F85-1108	38.9(39)	1.3(07)	30.6(34)	70.2(57)	41.5(40)
Kirby	32.4(35)	0.2(03)	8.2(17)	40.7(40)	67.2(55)
Mean	36.2	7.6	16.9	60.7	44.4
LSD (0.05)	(09)	(07)	(06)	(10)	(12)
Harvest time					
H_0	19.2(26)	5.4(13)	8.8(17)	33.5(35)	70.0(57)
H_1	39.8(39)	8.0(16)	17.7(25)	65.4(54)	38.8(39)
H_2	49.5(45)	9.3(18)	24.2(29)	83.1(66)	24.3(29)
LSD (0.05)	(05)	(03)	(06)	(08)	(09)

* arcsine $(\%)^{1/2}$ transformed means are presented in parentheses.

are shown in Table 2.5. Two means were calculated: one for the two genotype considered susceptible and another for the other three. Both sets of means show a consistent decrease in incidence of Colletotrichum from early to late maturity except for the abnormally high value of 10.0% for genotype F85-998 which matured 1 Oct., thus inflated the mean for the 09/26 - 10/03 period. As was the case for Phomopsis, however, there is the suggestion that infection increased slightly in seeds maturing in early November.

The incidence of seed with Colletotrichum was influenced significantly by time of harvest (Table 2.1). Seeds harvested at maturity averaged 5.4% incidence (Table 2.6), those harvested one week later averaged 8.0%, significantly more than 5.4%, and those harvested at two weeks after maturity averaged 9.3%, not significantly different from 8.0%. Effects of harvest date were consistent over genotypes and planting dates.

There was no effect of planting date on incidence of Fusarium in seeds. Also, maturity date apparently had little effect (Table 2.5). Genotypes differed significantly. The 30.6% incidence for F85-1108 was significantly more than for any other genotype. Kirby was lowest at 8.2% (Table 2.6).

Harvest time had a significant effect on incidence of Fusarium which was consistent over planting dates and genotypes (Table 2.1). Seeds harvested at maturity (8.8%) had lower Fusarium infection than seeds harvested one week

later (17.7%), and two weeks after maturity (24.2%) (Table 2.6).

Planting date influenced the incidence of seed with fungal infection (Table 2.1). The highest incidence (80.2%) was in seeds from the first planting date and there was a consistent decline in percentage of infection to 40.4% on planting 5, and a non-significant increase to 56.9% for date 6 (Table 2.5). This trend is similar to that for incidence of Phomopsis. Effects of maturity date are also shown in Table 2.5. The highest total incidence for all fungi occurred when plants matured between 18 Aug. and 19 Sept., a period of high temperatures and frequent rainfall. The lowest incidence occurred when plants matured between 11 and 27 Oct. and coincided with the lowest temperatures (Fig.2.2) and absence of rainfall from 11 through 24 Oct. (Fig.2.1). The increase in incidence of fungi on seeds that matured in early Nov. might be related to the rainy period from 25 to 30 Oct. and the increase in minimum temperature beginning about 24 Oct.

Genotypes also differed in incidence of seed with fungal infection (Table 2.1). Part of the lower incidence of Kirby (Table 2.6) was associated with seeds maturing during period less conducive to fungal infection (Table 2.3). The other four genotypes had higher fungal infection than Kirby and did not differ from each other.

The incidence of seed with fungal infection was influenced by harvest time. Seeds harvested at maturity averaged 33.5% infection, significantly less than those harvested one and two weeks later, 65.4 and 83.1%, respectively (Table 2.6).

Significant effect for planting date was observed for percentage of seed germination in blotter test (Table 2.1). The lowest seed germination was observed in PD_1 (28.3%) and PD_2 (32.2%), and there was a consistent increase to 61.2% in PD_5, and a slight non-significant decrease to 49.7% in PD_6 (Table 2.5). Highest germination occurred in seeds which matured between 11 and 27 Oct. and coincided with the environmental conditions less favorable for fungal infection. Lowest germination occurred in seeds which matured from 18 Aug. through 19 Sept., and was generally associated with high temperatures and frequent rainfall during this period. Fungal infection significantly reduced percentage of germination. It should be pointed out, however, that although percentages of seed germination from blotter tests are expected to be similar to those from the official rolled paper-towel method, they are often much lower than percentages obtained from germination in sand or tetrazolium tests, particularly when seed pathogens are the major cause of reduced germination (Franca Neto, 1989).

Genotypes differed in percentage of germination (Table 2.1). The 67.2% germination for Kirby was significantly more

than for any other genotype (Table 2.6). The other four genotypes did not differ from each other, ranging from 31.3 to 45.6% .

Harvest time had the largest effect on percentage of seed germination. Seeds harvested at maturity had higher germination (70.0%) than those harvested one or two weeks later, 38.8 and 24.3%, respectively (Table 2.6). The almost two-fold decrease from H_0 to H_1 and three-fold decrease from H_0 to H_2 confirmed the expectation that high infection due to harvest delay was detrimental to seed germination.

The severity of fungal infection and reduction in seed germination associated with time of maturity and delay in harvest in F85-994 and F85-1108 is shown in Table 2.7. Incidence of seed with fungi exceeded 100% for some early maturing plots, indicating that more than one fungal genus infected many seeds. Some infections probably were undetected. For F85-1108 in PD_2, percentage of seed germination was reduced from 50.0% at maturity to 0% with one week delay in harvest. The two genotypes have three common maturity dates among the six planting dates: 8/28, 9/26, and 10/13-14. F85-1108 was more adversely affected by harvest delay than F85-994 based on comparisons within these maturity dates. Even though the genotype x harvest date interaction was nonsignificant for percentage of seed germination (Table 2.1), there was a significant P x G x H effect which justifies making these comparisons.

Table 2.7. Effect of maturity date and harvest time on the percentage of total fungal infection of seed and seed germination in two genotypes in 1986

Harvest time	PD_1 5/6	PD_2 5/21	PD_3 6/17	PD_4 7/01	PD_5 7/15	PD_6 7/29	M
				F85-994			
Percentage total infection							
H_0	87.5	40.0	27.5	10.0	15.0	10.0	31.7
H_1	102.5	80.0	65.0	37.5	37.5	7.5	55.0
H_2	110.0	92.5	95.0	107.5	72.5	52.5	88.3
Percentage germination							
H_0	40.0	77.5	72.5	82.5	87.5	82.5	73.1
H_1	15.0	25.0	35.0	60.0	62.5	87.5	47.5
H_2	10.0	12.5	12.5	5.0	22.5	30.0	15.4
				F85-1108			
Percentage total infection							
H_0	32.5	55.0	30.0	40.0	10.0	50.0	36.3
H_1	87.5	115.0	105.0	67.5	35.0	60.5	78.4
H_2	110.0	117.5	115.0	105.0	47.5	90.0	97.5
Percentage germination							
H_0	70.0	50.0	77.5	72.5	97.5	62.5	71.7
H_1	12.5	0.0	12.5	37.5	75.0	55.0	32.0
H_2	0.0	0.0	0.0	5.0	80.0	40.0	20.8
Maturity date							
F85-994	8/18	8/28	9/19	09/26	10/03	10/14	
F85-1108	8/28	9/05	9/26	10/13	10/21	11/01	

1987 Results

For all variables measured in 1987, differences were found for planting date and harvest time and some significant interactions among factors (Table 2.8). Effects of genotype were nonsignificant. Effects of maturity date and harvest time for all variables are given in Table 2.9.

The highest incidence of Phomopsis on seed was in PD_1 (31.0%) followed by a lower level in PD_2 (14.2%), and 12.0% in PD_3. Very low incidence of Phomopsis was found for seeds of planting dates 4 through 6. These levels of infection were well below those observed in 1986. Probably, the factors which contributed to higher incidence in early planted plots in 1986 and 1987 were the same, however, the infection evidently was limited by the less frequent rainfall in 1987 (Fig.2.2). Similar to 1986, early planted plots matured earlier in the fall in 1987 and these plots also had longer life cycles (Table 2.10). Lower temperatures (Fig.2.2) and less rainfall (Fig.2.1) reduced infection in the later planted plots.

Maturity date was an important contributor to the high planting date x genotype interaction. High incidence of Phomopsis was found to occur from 31 Aug. to 17 Sept. From 7 to 31 Oct. the incidence was negligible but increased slightly in seeds produced in early November. As was the case for Phomopsis in 1986, the higher incidence in early maturing seeds was associated with high temperatures and

Table 2.8. Mean squares and F-tests on the incidence of fungal infection of seed and percentage of seed germination computed for experiments combined over planting dates in 1987.

Source of variation	d.f.	*Phomopsis* spp.	*C truncatum*	*Fusarium* spp.	Total infection	germi-nation
Planting date (P)	5	7226.1**	5145.3**	1618.2**	34666.4**	22146.9**
Genotype (G)	4	2193.0 ns	1435.2 ns	542.0 ns	10021.6 ns	5477.5ns
P x G	20	1001.2**	661.5**	223.1**	3828.5**	2255.8**
Harvest time	2	1682.8**	505.9**	539.4**	5938.1**	4991.3**
P x H	10	162.1*	63.2 ns	140.8**	602.6**	431.7**
G x H	8	87.6 ns	57.0 ns	65.0 ns	342.9 ns	172.9*
P x G x H	40	92.3 ns	61.7 ns	64.6 ns	294.0**	158.3**
Mean		10.3	7.2	4.2	21.7	80.5
C.V.		50.8	65.5	87.9	36.7	11.1

*, ** indicate significance at 5 and 1%, respectively.

Table 2.9. Effect of date of planting and maturity, and harvest time on the incidence of seeds with fungal infection and seed germination in 1987.

Planting date	Phomopsis spp.	C. truncatum	Fusarium spp.	Total infection	germination
			%		
PD_1	31.0(34)*	24.2(29)	9.8(18)	65.0(54)	38.5(38)
PD_2	14.2(22)	9.7(18)	6.3(15)	30.2(33)	73.8(59)
PD_3	12.0(20)	5.8(14)	5.0(13)	22.9(29)	79.3(63)
PD_4	0.9(5)	1.2(6)	2.0(8)	4.0(12)	98.0(82)
PD_5	2.0(8)	1.0(6)	0.9(5)	3.9(11)	96.6(79)
PD_6	1.7(7)	1.5(7)	0.9(5)	4.1(12)	96.8(80)
Mean	10.3	7.2	4.2	21.6	80.5
LSD (0.05)	(8)	(6)	(6)	(11)	(10)
Maturity date					
8/31-9/2	38.3	29.4	11.7	79.4	24.4
09/14-17	32.5	17.1	10.0	59.6	46.2
09/21-28	9.3	6.4	5.4	21.1	81.9
10/07	0.5	0.8	0.0	1.4	98.3
10/14-22	1.8	0.9	1.5	4.1	97.2
10/26-31	0.6	1.0	1.3	3.0	97.9
11/02-07	4.2	3.0	1.7	8.4	93.8
Harvest time					
H_0	6.3(15)	5.8(14)	2.6(9)	14.6(22)	87.6(69)
H_1	10.3(19)	7.1(15)	4.4(12)	21.7(28)	80.2(64)
H_2	14.3(22)	8.8(17)	5.5(14)	28.7(32)	73.7(59)
LSD (0.05)	(5)	(3)	(4)	(4)	(5)

* arcsine $(\%)^{1/2}$ transformed means are presented in parentheses.

Table 2.10. Dates of harvest maturity (R_8) and days to maturity in five soybean genotypes planted at six planting dates in 1987.

	PD_1*	PD_2	PD_3	PD_4	PD_5	PD_6
	4/30	6/03	6/11	6/30	7/17	7/24
F85-994	08/31 123	09/14 103	09/17 98	09/21 83	10/07 82	10/14 82
Forrest	09/02 125	09/23 112	09/24 105	10/14 106	10/19 94	10/26 94
F85-998	09/02 125	09/21 110	09/28 109	10/15 107	10/22 97	10/30 98
F85-1108	09/01 124	09/21 110	09/21 102	10/07 99	10/22 97	10/30 98
Kirby	10/16 169	10/26 145	10/29 140	10/31 123	11/02 108	11/07 106

* PD = planting dates.

more frequent rainfall during the period from 31 Aug. to 17 Sept.

Incidence of seed with Phomopsis consistently increased as harvest was delayed. Seeds harvested at H_0, H_1, and H_2 had 6.3, 10.3, and 14.3% infection, respectively, with H_0 differing significantly from H_2. These results are consistent with those from 1986, however the magnitude for 1987 was only about one-third that for 1986.

Plant genotype influenced the incidence of Phomopsis in seeds in 1986. When effects of genotype effects were excluded, weather condition immediately prior to or at maturity was important. As Alexander et al. (1978) suggested, the long-time average for weather conditions seems to be the best predictor for incidence of fungi for any planned maturity date.

Incidence of Colletotrichum was highest in seeds produced in PD_1 (24.2%) and there was a significant decline to 1.5% at PD_6. The incidence in PD_1 was higher than that observed in 1986. The incidence for the other planting dates, however, were lower. Effects of maturity date were similar to those observed in 1886. There was a consistent decrease in incidence from early to late maturity. Substantial infection by Colletotrichum can be expected if maturity occurs during conditions of high temperature and humidity.

There was a significant increase in incidence of Colletotrichum when harvest was delayed for 2 weeks. The magnitude of 5.8, 7.1, and 8.8% infection for H_0, H_1, and H_2, respectively, were similar to that of 1986 (Table 2.5).

The incidence of Fusarium in 1987 was completely different from that of the previous year. The incidence was moderate in the first three planting dates and decreased to very low levels in the other planting dates. This moderate infection, observed in seeds harvested from 31 Aug. to 17 Sept., was well below that for the same period in the previous year. Also, the higher incidence of Fusarium observed in F85-1108 in 1986 was not confirmed in 1987.

The incidence of fungal infection declined significantly from the first planting date (65.0%) to about one-half and one-third at PD_2 (30.2%) and PD_3 (22.9%), respectively, and was about 4% from PD_4 through PD_6. Compared to 1986, these levels were substantially lower, which may be attributed to the drier and cooler weather in 1987. The incidence of seed with fungal infection declined substantially in seeds that matured from 31 Aug. to 28 Sept., was negligible in seeds maturing in Oct., and increased slightly when maturity was from 2 to 7 Nov.

Harvest time had a significant effect on the incidence of seed with fungal infection. Seeds harvested at maturity averaged 14.6%, significantly different from seeds harvested one and two weeks later (21.7 and 28.7%, respectively).

Compared to 1986, effects of harvest time in 1987 were much less.

Percentage of seed germination was lower in PD_1 (38.5%) and significantly increased to 96.8% in PD_6. No significant differences were observed at the last three planting dates. Effects of maturity date were substantial. Lowest germination occurred in seeds that matured from 31 Aug. to 2 Sept. and increased steadily from 21 Sept. to 31 Oct., decreasing slightly from 2 to 7 Nov.

Seeds harvested at maturity had significantly higher germination (87.6%) than those harvested one week (80.2%) and two weeks (73.7%) later. This trend for 1987 was similar to that observed in 1986, however the effects of delay in harvest were substantially lower in 1987. Environmental condition from seed maturation to harvest was the major factor determining the level of fungal infection and the potential for seed germination. However, some effect of planting date, and the effect of planting date on the length of life cycle, may be important too.

Conclusions

1- The incidence of fungal infection and percentage of seed germination was influenced by the environmental conditions during the maturation period. Extensive fungal infection and

low percentage of germination occurred in seeds maturing under high moisture conditions.

2- Variable level of infection may be expected in seeds maturing at different dates within the same year or in different years. Results from 1986 and 1987 were different primarily due to a more moist season during seed maturity in 1986 and a drier season in 1987.

3- Phomopsis, Fusarium, and Colletotrichum were the three most important genera to infect soybean seeds in this study.

4- Infection by Phomopsis and Fusarium occurred at all maturity dates when seeds were subjected to frequent rainfall. Extensive infection by Colletotrichum occurred only in early maturing seeds subjected to high temperatures and heavy rainfall.

5- Planting date influenced amount of fungal infection of seeds and percentage of seed germination indirectly because it was partially confounded with maturity date. Early planted, early-maturing genotypes, which usually were harvested during periods of greater and more frequent rainfall, had the highest incidence of seed with fungal infection and the lowest percentage of seed germination.

6- Harvest time had the largest effect on fungal infection of seeds and on seed germination. The incidence of infected seeds increased consistently as harvest was delayed after maturity. Germination declined proportionally to the level of incidence.

7- For effective selection of resistance of seed to fungal infection, the most suitable maturity period seems to be predicted by the long-term average weather conditions.

CHAPTER III
INHERITANCE OF RESISTANCE TO INFECTION OF SOYBEAN SEED BY *Phomopsis* spp.

Poor quality soybean seed has been often associated with pathological, physiological, and mechanical causes. Most frequently it is the result of a combination of these factors. High relative humidity or rain and high temperature during and after physiological maturity, are the main factors associated with seed deterioration (Dassou and Kueneman, 1984; Hinson and Hartwig, 1977; Paschall II and Ellis, 1978; Tekrony et al., 1980). Several pathogens in combination with field weathering contribute to deterioration of soybean seed. Among them, the Phomopsis/Diaporthe complex is believed to be the most important in causing seed decay (Kmetz et al., 1974; Shortt et al., 1981; Tekrony et al., 1983). This fungal complex is known to cause pod and stem blight as well as seed decay. Included in this group are *Diaporthe phaseolorum* (Cke. & Ell.) Sacc. var. *sojae* (Lehman) Wehm, its anamorph *Phomopsis phaseoli* (Desm.) Sacc., and *Phomopsis longicola* Hobbs (Morgan-Jones, 1989). This group of fungi will be referred to herein as *Phomopsis* spp.

The disease is endemic to most soybean growing areas throughout the world. However, a high incidence of seed infection is achieved only with high temperature and high relative humidity (Balduchii and McGee, 1985; Tekrony et al., 1983). Kmetz et al.(1974) reported a great increase in seed infection by Phomopsis spp. from physiological maturity (R_7) to harvest maturity (R_8). Low incidences of Phomopsis have been reported in late-planting late-maturing soybean cultivars. The low incidence of Phomopsis seed decay in late-maturing genotypes was not due to inherent resistance but to escape from infection and disease development in two studies (Jordan et al., 1988; Wilcox et al., 1985).

The importance of Phomopsis spp. in reducing seed germination has been broadly reported. Phomopsis spp. did not affect emergence in soil (or sand) if seed were of high physiological quality and if environmental conditions resulted in fast emergence (Franca Neto, 1989; Henning and Franca Neto, 1985; Kulik and Schoen, 1981). Ndimande et al. (1981) conducted two experiments to determine whether the rapid loss of viability of soybean seed in lowland humid tropics was primarily due to physiological causes, pathological causes, or both. They suggested that seedborne fungi were important in seed deterioration prior to harvest, but much less important during storage.

Hardseededness has been considered a mechanism which may provide protection against weathering (Hartwig and

Potts, 1987) and infection by Phomopsis spp. (Hill et al., 1985; Yaklich and Kulik, 1987). Yaklich and Kulik (1987) also observed that infection by P. phaseoli and physiological deterioration were separate phenomena responsible for the loss of seed quality in soybean; however, they can operate in concert. They implied that impermeable seedcoat was an effective barrier to penetration by P. phaseoli.

Since physiological factors conducive to good quality in soybean are not yet well understood, plant breeders also concentrate on host resistance to seedborne pathogens as an adjunct mechanism to improve quality. Genetic variability for resistance to Phomopsis spp. has been reported by several authors. Current commercial varieties appear to differ little in this respect. New cultivars with improved seed quality are needed. Such cultivars would improve yield potentials in many traditional areas and would be even more important in tropical climates were high quality seed is a major factor limiting production. Paschal II and Ellis (1978) tested several genotypes to determine the extent of variation in the incidence of seed infection by fungi and its effect on seed viability under tropical conditions. They found substantial genetic variation for seed quality and incidence of Phomopsis spp. Many of the resistant strains were from Southeast Asia where environmental conditions cause considerable seed deterioration. Ndimande

(1981) also reported that two varieties of Indonesian origin were significantly less infected by Phomopsis spp. than were seeds of two varieties of U.S. origin. Berger and Hinson (1984) identified several breeding lines with a low incidence of seed infection by Phomopsis. PI 80837 had low seed infection in the Northcentral U.S., but was considered susceptible when grown in Southern U.S. Cultivars 'Delmar' and 'Ransom' have a high degree of resistance which appears to be heritable (Athow, 1987). PI 181550, introduced from Japan, also has been reported as having resistance. Yaklich and Kulik (1987) concluded that Sooty, a cultivar with a black and impermeable seedcoat, has some genetic resistance to seed infection. PI 417479 has exhibited a high level of resistance to Phomopsis seed decay when compared with 'Williams 82' and resistant genotypes like Delmar and PI 80837 (Brown et al., 1987). Brown and Minor (1986) gave a complete description of PI 417479. It was introduced from Japan and has high levels of anthocyanin in pod tissue at physiological maturity, as well as waxy globules on the surface of mature seeds. The breeding line F85-4901, selected from a breeding program for insect resistance, has good resistance to Phomopsis spp. When compared with other cultivars within the same maturity, plants of F85-4901 were more resistant to pod and stem blight disease when harvest was delayed (K. Hinson, personal communication).

Development of soybean cultivars resistant to Phomopsis spp. has been suggested as a long-term solution to the seed decay problem. Plant breeders are using the resistant genotypes described above as sources of resistance. However, there are no known reports on the inheritance of reaction to this disease. The primary objective of this study was to determine the genetic basis of resistance to Phomopsis infection of seed in PI 417479. A secondary objective was to obtain more information on the resistance qualities of F85-4901.

Materials and Methods

The soybean genotype PI 417479 carrying resistance to Phomopsis seed infection was crossed with five breeding lines in 1986 and with three cultivars in 1987. F85-4901 was crossed with Forrest in 1986. Crosses were made in the field at the Agronomy Farm, University of Florida, in Gainesville, Florida. The crosses involved in this study as well as the methods employed to favor natural infection of seed by Phomopsis spp. are shown in Table 3.1.

Three F_1 seeds from each 1986 cross were increased in an off-season nursery in Puerto Rico during the succeeding winter season. In mid-May 1987, the remaining F_1 seeds were spaced-planted 25 cm apart in single row plots 210 cm long and 91 cm apart. Additionally, F_2 seeds from the crosses

Table 3.1. List of crosses and methods employed for seed stress.

Cross	number	Weathering method
PI 417479 x F85-1138	1	delayed harvest
PI 417479 x F85-1235	2	delayed harvest
PI 417479 x F85-7260	3	delayed harvest
PI 417479 x F85-7352	4	delayed harvest
PI 417479 x F85-4901	5	delayed harvest
Forrest x F85-4901	6	delayed harvest
PI 417479 x 'Forrest'	7	incubator
PI 417479 x F85-4901	8	incubator
PI 417479 x 'Kirby'	9	incubator
PI 417479 x 'Doko'	10	incubator

increased in Puerto Rico and parental types were planted nearby in single row plots 600 cm long and 91 cm apart at a density of approximately 25 seeds m^{-1}. Cultural practices followed those recommended for commercial soybean production. Irrigation was used as needed to relieve moisture stress during the growth and pod-filling periods.

Field weathering was used for plants grown in 1987. Plants which reached harvest maturity near the same time were identified with colored plastic tape, a different color for each maturity date. This identification assured that plants harvested on the same date were subjected to similar environmental conditions. The F_2 populations were examined at weekly intervals and the plants within R_8 stage were tagged with tape. Plants were harvested 2 weeks later. Ten plants of each parental type and a variable number of F_1 plants were also tagged at harvest maturity. Harvested plants were scored for incidence of pod and stem blight, threshed individually in a single plant thresher, and the seeds were stored at room temperature ($\pm$ 21 ^{0}C, $\pm$ 60% RH) until incidence of infected seed was evaluated. Incidence of pod and stem blight disease was visually rated on a scale of 0 (no stem blight) to 5 (pycnidia abundant on 90% or more of the middle part of the plant).

The blotter test was carried out to evaluate the incidence of fungal infection of seed in the seeds harvested at different maturity dates. The procedure followed that

reported in Chapter II. A sample of 20 random seeds from each plant was used. The fungal species identified were Phomopsis spp., Colletotrichum truncatum (Schw.) Andrus and Moore, and Fusarium spp. Total seed infection was obtained by summing the incidence of infected seed with these three species. Germination was determined from the number of normal seedlings in the blotter-test plates.

F_1 seeds from the crosses performed in 1987 were increased in a greenhouse during part of the winter-spring seasons. In 1988, F_2 populations from the four crosses and parental types were grown in a similar mode to that of 1987. The segregating populations were examined at 3- to 4-day intervals and plants were promptly harvested at maturity. Ten plants of each parental type were also harvested at maturity.

A different procedure, incubator weathering, was used in 1988 to favor fungal infection of seeds in the F_2 and parental populations. A plastic chamber (incubator) was constructed inside a germinator room (25 ^{0}C, 85% RH). To condition high relative humidity, the chamber shelves were lined with two saturated germination paper towels. Additionally, trays on every second shelf, spaced 20 cm apart, were filled with water. Samples of 15 to 20 pods from each plant were placed on a wire mesh rack resting on a petri dish (14 cm x 2.5 cm) lined with two layers of water saturated blotter paper. The petri dishes with pods were

placed inside the incubator and were subjected to 25 ^{0}C and 100% relative humidity for 4 days. Pods were air-dried in a cold room (17 $\pm$ 1 ^{0}C, 65% RH) for 3 to 4 days, rated for incidence of pod and stem blight, hand-shelled, and the seeds tested for seedborne infection. The blotter test procedure was the same as for 1987.

Genetic hypotheses for one or two major genes for resistance was tested for goodness of fit by chi-square statistics. Data were transformed to arcsine square-root of percentage of seeds with Phomopsis infection and phenotypic, genotypic, and environmental variances, V_P, V_G, and V_E, respectively, were determined as:

$V_P = V_{F2}$, $V_G = V_{F2} - V_E$, and $V_E = (V_{P1} + V_{P2})/2$.

The broad-sense heritabilities (H^2) were calculated within crosses as $H^2 = V_G / V_P$ (Simmonds, 1979) assuming that environmental factors affected both segregating and parental genotypes to the same extent. The variability in the parental lines was also assumed to be entirely environmental, since they were homozygous. Phenotypic correlations were estimated as $r = COVxy / (Vx * Vy)^{1/2}$ (Falconer, 1985). The significance tests were performed at 95 and 99% levels of significance and n - 2 degrees of freedom, where n = number of observations.

Results and Discussion

Environmental conditions during the maturity-season in 1987 were not favorable to infection of seeds by Phomopsis as stated in Chapter II. Parents and F_2 plants from crosses weathered in the field had minimum opportunity for seed infection, particularly the late-maturing plants, despite being irrigated three times during the maturation period. Plants treated in the incubator in 1988 were more uniformly and highly infected. The 1988 results probably are better than those from 1987. For this reason, 1988 data will be presented first, followed by data from 1987 to see if these data support interpretations from the 1988 results.

Four F_2 populations and parents (crosses 7 - 10 in Table 3.1) were grown in 1988. The means and ranges are shown in Table 3.2. A large range in Phomopsis seed infection was detected in all F_2 population and in susceptible Forrest and Kirby.

Frequency distributions were drawn (Fig.3.1) to see if resistance can be regarded as a qualitative or quantitative character. Most of the crosses exhibited a multimodal distribution, but clear-cut phenotypic classes were not easily evident. F_2 populations exhibited substantial skewness toward the resistant parent, suggesting partial or complete dominance of gene(s) for resistance. Quantitative

Table 3.2. Mean and range of incidence of Phomopsis, total fungal infection, and percentage of seed germination for plants weathered in the incubator in 1988.

Population	n		Phomopsis infection	Total infection	germination
			%	%	%
		PI 417479			
P_1	10	Mean	9.5	14.6	87.
		Range	0 - 25	5 - 30	70 -100
		PI 417479 X Forrest			
F_2	69	Mean	38.8	46.9	55.2
		Range	5 - 95	5 - 100	0- 95
P_2	10	Mean	58.0	67.5	36.5
		Range	40 - 75	55 - 80	20 - 50
		PI 417479 X F85-4901			
F_2	44	Mean	33.5	46.9	52.5
		Range	5 - 80	5 - 100	0 - 95
P_2	10	Mean	36.0	41.5	61.0
		Range	15 - 60	15 - 65	40 - 85
		PI 417479 X Kirby			
F_2	142	Mean	34.0	42.4	59.0
		Range	5 - 75	5 - 90	10 - 100
P_2	10	Mean	56.0	68.5	36.0
		Range	40 - 85	45 - 100	15 - 55
		PI 417479 X Doko-ii			
F_2	116	Mean	12.0	16.4	67.0
		Range	0 - 80	0 - 95	5 - 95
P_2	10	Mean	15.0	19.1	83.0
		Range	0 - 30	5 - 30	70 - 85

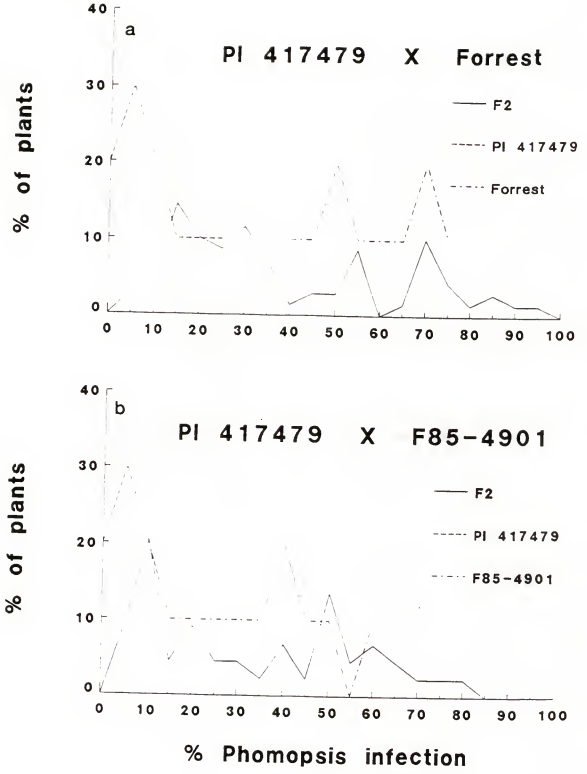

Fig.3.1. Frequency distribution of incidence of seed with Phomopsis infection in F_2 and parent plants.

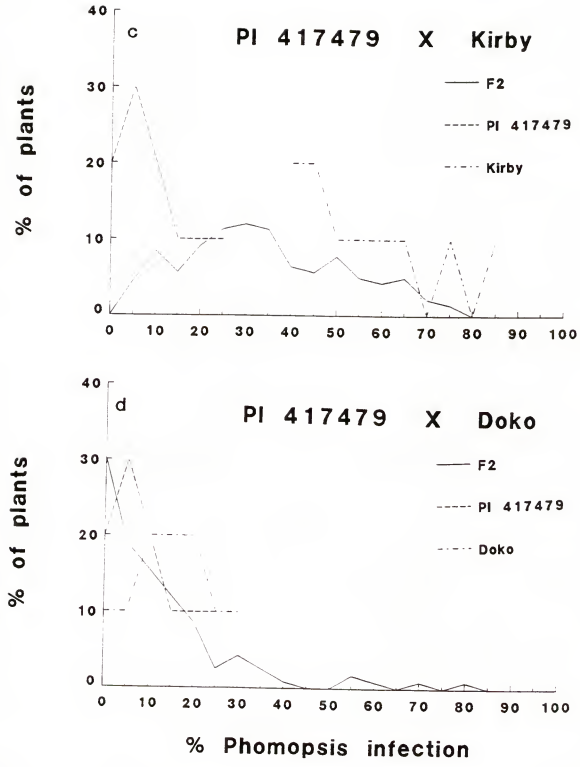

Fig.3.1. continued.

inheritance cannot be ruled out though, because transgressive segregation was apparent in several crosses.

F_2 plants with less than or equal to 30% of seeds infected by Phomopsis were classified as having resistance to infection. Plants with incidence greater than 30% were considered susceptible to seed infection by Phomopsis. Observed data for all F_2 populations and also chi-square values and probabilities to fit the appropriate genetic ratio are shown in Table 3.3.

The F_2's of cross 7 (PI 417479 x Forrest) exhibited a multimodal distribution with visible transgressivity for highly susceptible individuals (Fig. 3.1a). Forty-one plants were resistant and 28 were susceptible. There was a good fit to the 9:7 ratio with a low chi-square of 0.28 and $P > 0.50$. This segregation pattern suggests the existence of two dominant genes for resistance in PI 417479. Because of the relatively wide range, modifying genes might be important for the manifestation of high levels of resistance.

A similar pattern of segregation was observed in cross 8 between PI 417479 and F85-4901 (Fig. 3.1b). The observed frequency of 27 resistant and 17 susceptible plants is consistent with the expected 9:7 ratio with a low chi-square of 0.57 and $P > 0.25$.

No clear-cut categories were evident in Fig. 3.1c for cross 9 between (PI 417479 and Kirby) when all 142 plants were considered. However, there were two F_1 progenies and

Table 3.3. Observed values, chi-square values, and probabilities (P) for the genetic model of one and two dominant genes for resistance and heritability estimates (H^2).

Cross	n	R*	S*	Chi-square 3:1	Chi-square 9:7	P	H^2
			1988 (incubator)				
PI417479 x Forrest	69	41	28		0.28	.50-.75	0.71
PI417479 x F85-4901	44	27	17		0.57	.25-.50	0.62
PI417479 x Kirby	142	89	53		2.38	.10-.25	0.33
PI417479 x Doko-ii	116	?	?				
			1987 (field weathering)				
PI417479 x F85-1138	64	40	24		1.01	.25-.50	
PI417479 x F85-1235	74	53	21	0.45	1.17	.25-.50	
PI417479 x F85-7260	111	83	28	0.12		.50-.75	
PI417479 x F85-7352	65	46	19	1.03		.25-.50	
PI417479 x F85-4901	99	75	24	0.03		.75-.90	0.66
Forrest x F85-4901	166	120	46	0.65		.25-.50	

* R = resistant, S = susceptible

two harvest dates for each. Examination of the frequency distributions with nearly discrete classes suggested a tentative classification of 89 resistant and 53 susceptible plants. This observed ratio fit the 9:7 expected ratio with an acceptable chi-square of 2.38 and $P > 0.10$.

The frequency distribution of the cross 10 (PI 417479 x Doko-ii) followed a peculiar pattern (Fig. 3.1d). The great majority of F_2 plants were resistant, falling within the range of variation of both parents. Both parents may have the same major genes for resistance, with minor genes accounting for the low frequency of susceptible plants. Doko-ii is a selfblack seedcoat mutant in Doko, which consistently produces good quality seed with low incidence of Phomopsis (Franca Neto et al., 1985) in the tropical climate of "cerrado" region in Brazil. The inheritance of resistance in this cross remains unsolved, though.

Six F_2 populations were studied in 1987. Means and ranges are shown in Table 3.4. A large range in Phomopsis infection was detected on seeds of all F_2 populations and on seeds of susceptible Forrest. The resistant parent PI 417479 matured under conditions favorable for infection. Data in Table 3.4. confirm its resistance in the Gainesville environment. F85-4901 matured later when conditions were much less favorable for infection, therefore its apparent resistance in Table 3.4 may be misleading. Data from parents F85-1138, 1235, 7260, and 7352 were not available.

Table 3.4. Mean and range of incidence of Phomopsis, total fungal infection, and percentage of seed germination for plants weathered in the field in 1987.

Population	n		Phomopsis infection	Total infection	germination
			%	%	%
		PI 417479			
P_1	10	Mean	7.5	12.6	88.5
		Range	0 -25	0 - 35	75 -100
		Forrest			
P_2	10	Mean	55.0	77.5	26.5
		Range	35 - 80	50 - 100	5 - 50
		F85-4901			
P_2	10	Mean	6.0	11.1	93.0
		Range	0 - 15	0 - 20	85 -100
		PI 417479 x F85-1138			
F_2	64	Mean	20.9	31.7	71.1
		Range	0 - 85	0 - 100	0 - 100
		PI 417479 x F85-1235			
F_2	74	Mean	19.6	31.2	73.2
		Range	0 - 50	0 - 75	25 -100
		PI 417479 x F85-7260			
F_2	111	Mean	13.3	23.5	79.0
		Range	0 - 65	0 - 100	0 - 100

Table 3.4. continued.

Population			Phomopsis infection	Total infection	germi-nation
	n		%	%	%
		PI 417479 x F85-7352			
F_2	65	Mean	19.0	38.6	65.9
		Range	0 - 65	5 - 110	0 - 100
		PI 417479 x F85-4901			
F_1	07	Mean	13.0	23.0	80.0
		Range	5 - 20	15 - 40	65 - 90
F_2	99	Mean	18.7	36.8	65.7
		Range	0 - 70	05 - 100	0 - 95
		Forrest x F85-4901			
F_1	05	Mean	1.1	2.2	98.0
		Range	0 - 5	0 - 5	95 -100
F_2	166	Mean	34.5	42.5	67.3
		Range	0 - 85	0 - 100	5 - 100

The incidence of Phomopsis was not consistent over the different groups of maturation in each cross. For this reason frequency distributions are shown separately in Table 3.5 for each group of plants which matured near the same date. Plants were tentatively separated into resistant and susceptible classes based on break-points in the frequency distribution and the mean infection level for each harvest date.

In the cross 1 (PI 417479 x F85-1138) there were a total of 64 F_2 plants tentatively classified as 40 resistant to 24 susceptible, an acceptable fit to the 9:7 ratio with a chi-square of 1.01 and $P > 0.25$. This result is similar to that observed in some crosses subjected to incubator weathering in 1988.

In cross 2 (PI 417479 x F85-1235) there were 74 F_2 plants. Thirty seven were considered resistant, 21 were considered susceptible, and 16 were intermediate. By including the 16 plants with intermediate reaction in the resistant category, a 3 resistant to 1 susceptible ratio was obtained (chi-square= 0.45, $P >.25$). If the intermediate plants were considered susceptible, the results fit a 9:7 ratio (chi-square= 1.17, $P > 0.25$). The most susceptible plants in this cross had about half the infection level as the most susceptible plants in the cross PI 417479 X F85-1138, suggesting a genetic difference between F85-1235 and

Table 3.5. Frequency distribution of F_2 plants which matured near the same date in 1987, number plants (n), and mean incidence of seeds with Phomopsis.

Harvest date	% Phomopsis infection															
	0	5	10	15	20	25	30	35	40	45	50	55	60	>65	n	mean
PI 417479 X F85-1138																
10/03	1			3		2	/*	1		1			3	1	12	36.7
10/07	1	2	2	5	1	/	3		1		1	1		2	19	26.0
10/10	7	2	2	4/	3					1		1		1	21	15.7
10/18	5	3/	2	1	1										12	5.8
PI 417479 X F85-1235																
10/03	3	4	6	1	2	1/	5		2	2	1				27	19.6
10/10	17	7/	2	2	2		1								31	5.0
10/18	6	6/	4												16	4.4
PI 417479 X F85-7260																
10/03	5	3	6	3	3	2	1	/	3		1	1	1		29	18.8
10/07	4	7	10	5	4	2	/	2		1	2		1		38	16.3
10/10	19	12/		3	4	3	1			2					44	7.2
PI 417479 X F85-7352																
10/03	3	5	7	3	3	3	1/	2	2	2		1			32	18.9
10/07	4	5	6	6	/	2	2	3	1	2	1		1		33	19.1

Table 3.5. continued

Harvest date	% Phomopsis infection															
	0	5	10	15	20	25	30	35	40	45	50	55	60	>65	n	mean
PI 417479 X F85-4901																
10/07	2	6	7	6	6	4	6/	2	1			2	1	2	45	22.3
10/12	4	7	1	/	3	1			1	1	2	2			22	19.3
10/23	6	8	4	6	2/	3	1	1						1	32	13.1
Forrest X F85-4901																
10/23	7	12	6	5	1	5	5	5	1/	4	3	4	2	11	71	30.8
11/07	1	2	1	3	5	5	4	6	9/	4	3	1	2		46	36.5
11/11			1	4	6	2	4	8	7	6/	1	4	3	3	49	37.9
PI 417479 X Kirby																
10/10		1	1	2	2	7	4	3	1	/	3		2	4	30	35.5
10/21		2	3	2	3	1	6	3	2	1/	4	2	1	5	35	36.1
10/10			4	2	4	4	4	4	3	3/	3	5	3	3	42	37.7
10/21		4	4	2	4	4/	3	6	3	4	1				35	26.3

* / = cut-point to separate between resistant and susceptible classes.

F85-1138. However, the inheritance of resistance in cross PI 417479 X F85-1235 remains unsolved.

The frequency distributions for cross 3 (PI 417479 x F85-7260) and cross 4 (PI 417479 x F85-7352) fit a 3:1 ratio best, suggesting that a single dominant gene might control resistance in both crosses. Both breeding lines have impermeable seedcoat, thus the populations were segregating for hardseededness. Hill and West (1982) demonstrated that fungi can penetrate seeds through pores on the seedcoat surface, implying that impermeable seedcoat can be an effective barrier to penetration and infection by P. phaseolorum (Yaklich and Kulik, 1987). In this study, hardseeded F_2 plants were as infected as non-hardseeded types, thus hardseed apparently had no effect on segregation ratios. The trait only protects against late infection which takes place after harvest maturity.

Cross 5 (PI 417479 x F85-4901), resistant x moderately resistant, produced a good fit to the 3:1 ratio. From the 1988 data, resistance may be controlled by two dominant genes. Escape from seed infection may have caused misclassification in 1987. Cross 6 between Forrest and F85-4901 also produced results too ambiguous to determine genetic control of resistance in F85-4901, despite the good fit to the 3:1 ratio. However, the wide range of seeds infected by Phomopsis within each harvest date may indicate

the presence of a moderately large genetic difference in resistance among F_2 progeny.

Two major dominant genes may be involved in the resistance of PI 417479 to infection of seeds by Phomopsis. The failure to obtain discrete resistant and susceptible classes also may indicate the presence of modifying genes.

Broad-sense heritability was estimated for each population grown in 1988 (incubator weathering) and for one population in 1987 (field weathering). Estimates are presented in Table 3.3. The moderate to high broad-sense heritability estimates indicated the populations contained substantial genetic variability for resistance to Phomopsis infection of seeds. The lowest estimate ($H^2 = 0.33$) in cross 9 was caused primarily by the rather high variance of the parent Kirby which reduced the ratio of genotypic variance to the total variance. The highest estimate ($H^2 = 0.71$) was for cross 7 having PI 417479 and Forrest as parents.

These relatively high broad-sense heritability indicate that effective selection for resistance to Phomopsis infection is possible in segregating generations. A conventional breeding procedure, such as pedigree selection, in concert with an appropriate method to favor infection of seed, like incubator weathering, would be effective to improve resistance to Phomopsis seed decay. However, high levels of resistance, through the cumulative effect of

modifying genes, would be better achieved by using recurrent selection procedures.

A strong relationship of Phomopsis infection of seeds with another variable more easily identified or quantified would be of extreme value during the selection procedure to improve the quality of soybean seed. Correlations among stem blight and pod blight ratings, incidence of Phomopsis, incidence of fungi, and percentage of seed germination in blotter test were calculated for the various populations. Phenotypic correlation estimates are presented in Table 3.6. Correlation estimates of ratings of stem blight disease with incidence of seeds with Phomopsis, incidence of seeds infected by fungi, and percentage of seed germination were inconsistent among crosses and in general of small magnitude. Ratings of pod blight rate were significantly correlated with these three variables, but of low practical importance because of their small magnitude. A strong negative correlation was found to exist between incidence of Phomopsis and percentage of seed germination in the blotter test. Numerous other studies have recognized this inverse relationship between incidence of Phomopsis and percentage of seed germination in the rolled-paper-towel (Franca Neto, 1989) and on culture media (Wilcox et al.,1985). The associations between incidence of fungi vs. percentage of seed germination and incidence of Phomopsis vs. percentage of seed germination were highly significant and of great

Table 3.6. Phenotypic correlation estimates between traits associated with soybean seed in several crosses

Cross	Stem blight rate vs			Pod blight rate vs.			Phom.+ x Germ.	TI++ x Germ.
	Phom.	TI	Germ.+++	Phom.	TI	Germ.		
	%							
1	0.31**	0.36**	-0.33**	0.61**	0.66**	-0.64**	-0.98**	-0.99**
2	0.41*	0.43**	-0.43**	0.66**	0.70**	-0.68**	-0.91**	-0.96**
3	0.39**	0.44**	-0.40**	0.37**	0.35**	-0.33**	-0.92**	-0.98**
4	0.22*	0.29**	-0.30**	0.38**	0.47**	-0.43**	-0.86**	-0.99**
5	0.04ns	0.18ns	-0.15ns	0.36**	0.45**	-0.43**	-0.90**	-0.99**
6	0.22**	0.24**	-0.23**	0.56**	0.57**	-0.50**	-0.93**	-0.93**
7				0.42**	0.48**	-0.39**	-0.89**	-0.90**
8							-0.95**	-0.97**
9				0.34*	0.49**	-0.45**	-0.70**	-0.75**
10				0.52**	0.52**	-0.08**	-0.05ns	-0.01ns

+ Phom. = Phomopsis ++ TI = Total infection +++ Germ. = germination.
*,** significantly different from zero at the 5 and 1% levels.

magnitude (Table 3.6). These strong associations can be of practical significance for breeding programs directed to improve seed quality through the use of resistance. From the breeders point of view, the identification and evaluation of seedborne pathogens are the bottleneck in the selection procedure. An alternative to this slow routine test would be to evaluate the percentage of germinated seedlings in the blotter test as an estimate of the incidence of Phomopsis. This decision could be taken after checking that other fungi are occurring in a low frequency. The effectiveness of selection would be increased probably as a result of two components: (i) through the concomitant selection for resistance to pathological and physiological causes of seed deterioration; and (ii) through the increase of population and sample sizes to be assessed.

Conclusions

The resistance to Phomopsis infection of seeds in PI 417479 is likely qualitative, resistance is dominant, and modifying genes may be present to obscure clear-cut segregation ratios. Although not conclusive, the data support the hypothesis for two major genes with 9:7 segregation ratios in F2 populations generated from crosses with susceptible genotypes. Doko-ii displayed resistance qualities in the Gainesville environment similar to those

displayed in Brazil. The genetic basis for resistance appears similar to that for PI 417479. F85-4901 probably has a different mechanism for resistance. Broad sense heritability estimates were moderately high indicating that high resistance can be obtained by using appropriate breeding procedures. Owing to the high correlation between incidence of seeds with Phomopsis and percentage of seed germination, breeders could select for percentage of seed germination in the blotter test as an alternative screening procedure to improve general pathological and physiological resistance.

CHAPTER IV
INHERITANCE OF BROWN RADICLE PATTERN IN THE SOYBEAN SEEDCOAT

Soybean [Glycine max (L.) Merr.] exhibits considerable variability in seedcoat pigmentation and pattern. The inheritance of different color pigments and patterns has been summarized by several authors (Williams, 1952; Johnson and Bernard, 1963; Bernard and Weiss, 1973; Palmer and Kilen, 1987). Seed colors are yellow, green, gray, black, imperfect black, brown, buff, reddish-brown, and reddish-buff. Saddle patterns occur in yellow, green, and tan seedcoat, while brown flecks occur in black and imperfect black seedcoat. Another distinct phenotype which occurs only in a few cultivars is the presence of brown and black stripes in a semicircular pattern (concentric brown and black rings) around the hilum. Most of the commercially grown grain cultivars are yellow-seeded, but a few have a black or green seedcoat which is considered objectionable for certain commercial uses of the soybean. Hilum color and seedcoat color, in addition to flower and pubescence colors, are widely used as marker characteristics to distinguish

between hybrids and self-pollinated progenies, when making artificial crosses (Specht and Williams, 1978; Palmer and Stelly, 1979).

Williams (1952) was the first to review the earlier studies about the inheritance of pigment color in soybean seed. Based on his experiments, he compared and clarified the somewhat divergent genetic interpretations and genetic symbols assigned to the various loci for seedcoat and hilum colors. The confusion among early investigators was caused by the pleiotropic effect of genes governing pubescence color and the epistatic effect of genes governing flower color.

Green seedcoat is controlled by a single completely dominant **G** gene. Two other complementary recessive genes, $\mathbf{d}_1$ and $\mathbf{d}_2$, produce seeds which are green throughout, including cotyledons, embryo axis and seedcoat. Also, chlorophyll retention is evident in other parts of the plant. Leaves and pods fail to turn yellow during normal senescence (Woodworth, 1921). Another distinct genetic factor for maternal inheritance, cyt-**G** , described by Terao (1918), causes the same phenotype described by Woodworth.

Two allelomorphic series were identified in soybeans. One allelic series with three alleles and one independent locus govern color and distribution of pigment in the seedcoat. The allelic series, genes **R**, $\mathbf{r}^{\mathbf{m}}$, and **r**, control the color pigment and concentric-ring pattern. The other

independent locus, **oo**, causes a self reddish-brown seedcoat. Black seedcoat or hilum (**R-**) is completely dominant to brown (**rr**) with brown epistatic to reddish-brown (**oo**) (Nagai, 1921; Williams, 1952; Bhatt and Torrie, 1968). The gene for tawny pubescence (**T**) has a pleiotropic effect on seed color, and is complementary with **R** to form the intense black color (Woodworth, 1921). Also, white flower gene w_1 is epistatic to **R** in gray pubescent genotypes **t**, changing the seedcoat color from imperfect black to buff (Stewart, 1930; Mahmud and Probst, 1953). Black concentric striping (r^m) was found to be dominant to brown and recessive to black (Weiss, 1970).

The other allelic series with four alleles controls the distribution of pigment in the seedcoat. Gene **I** prevents the normal development of pigment, i^i restricts pigment to the hilum, i^k produces a saddle pattern, and **i** is responsible for self-pigmented seedcoat (Williams, 1952; Mahmud and Probst, 1953). Bhatt and Torrie (1968) reported the sequential order $I > i^i > i^k > i$ for nearly complete dominance, but in certain crosses, distinct heterozygotes and reversal of dominance were identified. The **I** gene for light hilum produced yellow hilum with **Tr**, tRw_1, and **tr** genotypes, and gray hilum with **TR** and tRw_1 genotypes.

Three more genes at different loci are known to cause a saddle pattern similar to that of i^k. Williams (1958) reported a mutation for black or brown saddle to be due to a

single recessive gene. This mutation was later found to be the same recessive gene **kk** reported by Takagi (1929). A different kind of saddle pattern controlled by a single recessive gene designated k_2, was reported by Rode and Bernard (1975). The k_2 gene always produces a tan saddle, and is independent of the effects of the hilum color genes. Another mutant, with black or brown saddle, non-allelic to i^k, k_1, and k_2, has been assigned the gene symbol k_3 (Bernard and Weiss, 1973).

Black seedcoat with brown flecks has been reported to be controlled by a single dominant gene, designated **Fl** (Morse and Cartter, 1937). The relationship of the **Fl** gene to other genes affecting pigmentation in the seed was studied by Buzzell (1985). He found that the gene **R** is needed for the expression of flecked brown-black seed. However, the W_1 gene (purple flower), which affects the black pigmentation, is not needed for the expression of **Fl** gene, but the buff seedcoat must be examined before the seed dries at maturity to observe the flecking pattern accurately.

A new phenotype for pattern in the seedcoat was found at the National Center for Soybean Research (CNPS-EMBRAPA), Londrina, Parana, Brazil, in 1983. This new phenotype has a conspicuous brown pigmentation confined to the seedcoat region corresponding to the radicle-hypocotyl axis. This

trait herein called brown radicle has been identified only with light or gray hilum.

The objectives of this study were to determine the inheritance of brown radicle pattern and to investigate its relationship with other loci controlling pigment formation in soybean.

Materials and Methods

The genotypes used in crosses are described in Table 4.1. The brown radicle breeding lines BR83-18293, 18295, and 18297 were selected from the cross BR80-3621 x 'UFV-1'; BR85-3010 was selected from the cross BR80-3621 x 'Bossier'. The breeding line BR80-3621 originated from a multicross having 'Davis', 'Viçoja', 'Santa Rosa', and 'Kanrich' as parents to incorporate multiple resistance to several diseases in soybean.

To study the inheritance of brown radicle pattern several crosses were performed in the summer of 1986. Crosses were made in the field at the Agronomy Farm, University of Florida, Gainesville, FL. Parents were planted in blocks replicated over time to facilitate matching of flowering, since the genotypes with brown radicle pattern were late maturity. The hybrid populations are described in Table 4.2.

Table 4.1. Origin, genotype, and description of parents used to study the inheritance of brown radicle pattern.

Parent	Origin	Genotype*	Seed phenotype	Maturity group
BRF87-174[+]	BR83-18293[++]	W_1TI	gray seedcoat brown radicle	X
BRF87-193	BR83-18295	W_1TI	gray seedcoat brown radicle	X
BRF87-195	BR83-18297	w_1TI	gray seedcoat brown radicle	X
BRF86-03	BR85-3010A	w_1TtI	green seedcoat brown radicle	X
Forrest	USA cultivar	w_1Ti^i	yellow seedcoat black hilum	V
Kirby	USA cultivar	W_1Ti^i	yellow seedcoat black hilum	VIII
Centennial	USA cultivar	W_1Ti^i	yellow seedcoat black hilum	VI
Ogden	USA cultivar	W_1TI	green seedcoat light hilum	VI
Davis	USA cultivar	w_1ti^i	yellow seedcoat buff hilum	VI

*W_1 = purple flower; w_1 = white flower; T = tawny pubescence; t = gray pubescence; I = light hilum ; i^i = dark hilum

[+]BRF = symbol adopted for lines grown in Gainesville, from lines selected in Brazil at CNPS-EMBRAPA.

[++]BR = Symbol for breeding lines at CNPS-EMBRAPA,Londrina.

Table 4.2. Crosses made to study the inheritance of brown radicle pattern.

Cross	Cross number	F_1 genotype*
BRF87-174 x Kirby	1	WWTTIi^i
BRF87-194 x Forrest	2	WwTTIi^i
BRF87-195 x Centennial	3	WwTTIi^i
BRF86-03 x Ogden	4	WwTtII WwttII
BRF86-03 x Davis	5	wwTtIi^i wwttIi^i

* F_1 plants were homozygous for black hilum (**RR**)

The F_1 seeds were space-planted 30 cm apart in rows 210 cm long and 91 cm apart. A sudden incidence of lesser cornstalk borer [Elasmopalpus lignosellus (Zeller)] caused severe damage to the spaced F_1 plants. Crosses were again made in 1987. The F_1 plants from these seeds were planted in the greenhouse during part of the 1988 winter-spring seasons. The light period was extended to 15 hours for 3 weeks after seedling emergence to avoid early flowering conditioned by short photoperiod. At maturity, F_1 plants were individually harvested, threshed, and classified for seed color pattern.

The parents (Table 4.1) and F_2 populations from the crosses listed in Table 4.2 were grown in the field at Gainesville, Florida, in 1988. The F_2 seeds were drill planted in rows 600 cm long, with 91 cm between rows. The F_1 progenies from each cross were kept separate to identify selfing and determine homogeneity among the progenies. For each cross, three to four families were planted in adjacent rows with a density of 30 seeds m^{-1} to have a minimum of 100 F_2 plants per progeny at harvest.

Cultural practices followed those recommended for commercial soybean production in Florida. Weeds were controlled with the herbicide alachlor (2-chloro-2',6'-diethyl N-(methoxymethyl)-acetanilidine) complemented with mechanical cultivation. Fertilization was based on soil analysis. The area was an Arredondo fine sandy soil, a

loamy siliceous hyperthermic, Grossaremic Paleudult. Sprinkler irrigations were applied as needed to relieve water stress.

Mature F_2 plants were harvested weekly, classified for pubescence color and threshed separately. In the laboratory, hilum color and presence or absence of brown radicle were visually determined on seeds from F_2 plants. Four distinct categories were established. The phenotypic categories, with absence of brown radicle considered normal, were: i) normal seedcoat and light hilum; ii) normal seedcoat and dark hilum; iii) brown radicle and light hilum; and iv) brown radicle and dark hilum. Light hilum ($I_$) types were either a gray, green, or yellow color, which contrasted with black hilum (i^iR) types.

Standard chi-square tests for goodness of fit (Snedecor and Cochran, 1980) were used to test the genetic hypotheses. Chi-square tests for homogeneity were used to test homogeneity within crosses (among F_1 progenies) and among crosses.

Results and Discussion

Brown radicle pattern was absent in seeds produced by F_1 plants. This suggests that inheritance is recessive. However, in a few seeds a small amount of brown pigment, limited to the micropyle, was observed.

The expression of brown radicle varied in seeds from F_2 plants. In some cases it was dark brown, similar to the parents, whereas on other seeds there was only a tinge of brown. In the later cases, however, pigmentation was distinctly different from the brown speck on the micropyle observed in a few seeds from F_1 plants. All F_2 seed lots with the brown radicle, whether dark or faint, were grouped in one category for testing hypotheses on mode of inheritance. Data in Table 4.3, to test for goodness of fit to the ratio 3 normal: 1 brown radicle, are presented by crosses and F_1 progeny within crosses.

The overall ratio of 760 normal plants to 253 brown radicle gives a perfect fit to the 3:1, even though data from cross 4 fits the alternative 13:3 ratio better. Cross 2 also gives an acceptable fit to the 13:3 ratio but fits the 3:1 ratio better (chi-square= 0.41 vs. 2.99). Data from the other three crosses do not fit the 13:3 ratio. The generally good fit of data from each cross to the 3:1 ratio and the perfect overall fit is considered sufficient evidence to conclude that brown radicle pattern is inherited as a simple recessive gene. Gene symbol **rd** is proposed.

Progeny tests of F_2 plants will be conducted to support or reject the above conclusion. In addition, they are expected to provide information on whether the dilute expression of brown radicle was environmentally induced (e.g. plants damaged by whitefly) or if modifying genes

Table 4.3 Observed and expected 3 normal:1 brown radicle phenotypic ratios in five F_2 populations.

F_1 progeny	n	normal seedcoat	brown radicle	Chi-square	Chi-square probability
		CROSS 1 (BRF87-174 X KIRBY)			
1	67	46(50)*	21(17)	1.44	.10-.25
2	84	62(63)	22(21)	.06	.75-.90
3	67	47(50)	20(17)	.08	.75-.90
Pooled	218	155(164)	63(54)	1.77	.10-.25
Homogeneity				.18	.90-.95
		CROSS 2 (BRF87-194 X FORREST)			
1	82	64(62)	18(20)	.41	.50-.75
2	66	50(50)	16(16)	.02	.75-.90
3	85	65(64)	20(21)	.10	.75-.90
Pooled	233	179(175)	54(58)	.41	.50-.75
Homogeneity				.11	.90-.95
		CROSS 3 (BRF87-195 X CENTENNIAL)			
1	74	56(55)	18(19)	.02	.75-.90
2	80	55(60)	25(20)	1.67	.10-.25
3	41	29(31)	12(10)	.40	.50-.75
Pooled	195	140(146)	55(49)	1.07	.25-.50
Homogeneity				1.16	.50-.75

Table 4.3 continued.

F_1 progeny	n	normal seedcoat	brown radicle	Chi-square	Chi-square probability
		CROSS 4 (BRF86-03 X OGDEN)			
1	57	46(43)	11(14)	.99	.25-.50
2	71	55(53)	16(18)	.23	.50-.75
3	54	44(40)	10(14)	1.21	.25-.50
4	53	43(40)	10(13)	1.06	.25-.50
Pooled	235	188(176)	47(59)	3.13	.05-.10
Homogeneity				.36	.90-.95
		CROSS 5 (BRF86-03 X DAVIS)			
1	55	40(41)	15(14)	.15	.50-.75
2	33	24(25)	9(8)	.09	.75-.90
3	44	34(33)	10(11)	.12	.50-.75
Pooled	132	98(99)	34(33)	.04	.75-.90
Homogeneity				.32	.75-.90
		COMBINED OVER FIVE CROSSES			
Polled	1013	760(760)	253(253)	.0	>.95
Homogeneity				6.42	.10-.25

* Expected numbers are in parentheses.

influence expression. In this study gene **T** for tawny pubescence appeared to intensify brown radicle pigmentation. Gene W_1 for purple flower may have influenced pigmentation also.

From crosses of brown radicle parents with Kirby, Forrest, and Centennial, it was possible to investigate if the **I** and **rd** loci segregated independently. From F_2 data in Table 4.4 it was observed that light hilum (**I_**) was dominant to dark hilum (i^li^l), which is consistent with earlier reports (Bhatt and Torrie,1968). Also, in this study brown radicle pattern was determined to be controlled by the recessive alleles **rd rd.**

Data from crosses 1, 2, and 3 (Table 4.2) are summarized in Table 4.4 to provide information on the genetic relationship between the Ii^l and **Rd rd** loci. Observed values are shown along with expected values for the 9:3:3:1 ratio assuming independent assortment. The chi-square analysis showed a very poor fit to 9:3:3:1 ratio, primarily because of a complete absence of the double recessive phenotype and an excess of the **I_ rdrd** phenotype.

Three hypotheses were examined. The first, that **rd** was an additional allele in the **I**, i^k, i^l, **i** multiple allelic series, was discarded because three phenotypes were observed. The multiple allele hypothesis allows only two phenotypes in this crosses.

A second hypothesis examined is that i^I is epistatic to **rd**, thus preventing **rd** from producing pigment in the radicle-hypocotyl axis. This hypothesis can be supported by a good fit to a 9:3:4 ratio.

A third hypothesis is a close linkage between genes I and **rd**, which entered crosses in the repulsion phase. This hypothesis would be confirmed by obtaining a very low frequency of i^I**rd** phenotypes which presumably could arise only from recombination of two crossover gametes. It would be supported by a good fit to a 2:1:1: ratio based on Morgan's principle for nonindependent assortment of genes.

Table 4.5 presents data to distinguish between the epistasis and close linkage hypotheses. In two of the three crosses and in combined data the observed phenotypes gave a poor fit to the 9:3:4 ratio. In all cases, observed data fit the 2:1:1 ratio very well. Therefore, the epistasis hypothesis is rejected and the close linkage hypothesis is tentatively accepted, pending a search for crossover types in F_3 progeny. Locus **I** along with two other loci belong to Linkage Group VII. The order of genes was shown to be Y_{13}- **o - i** (Weiss,1970). Gene Y_{13} causes chlorophyll deficiency and **o** governs reddish-brown seedcoat. They could be used to determine if **rd** locus is a member of Linkage Group VII.

Table 4.4. Observed and expected phenotypic 9:3:3:1 ratios in F_2 plants for independent segregation between brown radicle **rd** and light hilum **I**

F_1 Progeny	n	I_ Rd_	i^i Rd_	I_ rd	i^i rd	Chi-square	Chi-square probability
			CROSS 1 (BRF87-174 X KIRBY)				
1	67	31(37)*	15(13)	21(13)	0(4)	8.73	.01-.05
2	84	39(47)	23(16)	22(16)	0(5)	12.51	<.01
3	67	31(37)	16(13)	20(13)	0(4)	10.72	.01-.05
Pooled	218	101(121)	54(42)	63(42)	0(13)	29.44	<.01
Homogeneity						2.51	.25-.50
			CROSS 2 (BRF87-194 X FORREST)				
1	82	43(46)	21(15)	18(15)	0(5)	7.72	.05-.10
2	66	31(37)	19(12)	16(12)	0(4)	9.74	.01-.05
3	85	47(48)	18(16)	20(16)	0(5)	6.63	.05-.10
Pooled	233	121(131)	58(44)	54(44)	0(15)	22.46	<.01
Homogeneity						1.64	.25-.50

Table 4.4. continued.

F_1 progeny	n	I_ Rd_	i^i Rd_	I_ rd	i^i rd	Chi-square	Chi-square probability
			CROSS 3 (BRF87-195 X CENTENNIAL)				
1	74	36(41)	20(14)	18(14)	0(5)	9.32	.01-.05
2	80	41(45)	14(15)	25(15)	0(5)	7.09	.05-.10
3	41	18(23)	11(8)	12(8)	0(3)	7.52	.05-.10
Pooled	195	95(110)	45(36)	55(36)	0(12)	25.40	<.01
Homogeneity						1.48	.25-.50
			COMBINED OVER THREE CROSSES				
Pooled	646	317(363)	157(121)	172(121)	0(41)	78.30	<.01
Homogeneity						1.00	.50-.75

* Expected numbers are in parentheses.

Table 4.5. Expected and observed phenotypic ratios in F_2 seeds of three crosses testing the alternative hypothesis of linkage and epistasis between genes controlling light hilum color **I** and brown radicle **rd**.

n	Expected ratio	I- --- Rd_	I_ ---- rdrd	i†i† ---- Rd_	Chi-square	Probability
		CROSS 1 (BRF87-174 X KIRBY)				
218		101	63	54		
	E+ 9:3:4	123	41	54	15.79	<.01
	L++ 2:1:1	109	54	54	1.91	>.30
		CROSS 2 (BRF87- 194 X FORREST)				
233		121	54	58		
	E 9:3:4	131	44	58	3.21	.20
	L 2:1:1	116	58	58	.48	>.75
		CROSS 3 (BRF87-195 X CENTENNIAL)				
195		95	55	45		
	E 9:3:4	109	37	49	11.55	<.01
	L 2:1:1	97	49	49	1.14	>.50
		COMBINED OVER THREE CROSSES				
646		317	172	157		
	E 9:3:4	363	121	161	27.41	<.01
	L 2:1:1	323	162	162	.92	>.50
		HOMOGENEITY				
	E				3.14	>.10
	L				2.62	>.25

†E = epistasis ††L = linkage

Data from crosses 4 and 5 provided information on the genetic relationship between the **T** (pubescence color) and **rd** loci. Observed values along with expected values for the 9:3:3:1 are shown in Table 4.6. Chi-square analysis showed a good fit to the 9:3:3:1 ratio. Complete independence was found between genes for pubescence color and brown radicle pattern.

Conclusions

Brown radicle pattern was found to be controlled by a single recessive gene. Gene symbol **rd** is proposed. Expression of **rd** may be influenced by modifying genes. A close linkage between the **I** and **rd** loci was tentatively accepted, pending a search for crossover in F_3 progeny. Complete independence was found between pubescence color **T** and brown radicle pattern **rd** loci.

Table 4.6. Observed and expected phenotypic 9:3:3:1 ratios in F_2 plants for independent segregation between brown radicle **rd** and pubescence color **T** loci

F_1 progeny	n	T_ Rd_	t Rd_	T_ rd	t rd	Chi-square	Chi-square probability
			CROSS 4 (BRF86-03 X OGDEN)				
1	54	33(31)*	11(10)	8(10)	2(3)	1.31	.50-.75
2	53	29(30)	14(10)	7(10)	3(3)	2.58	.25-.50
Pooled	107	62(60)	25(20)	15(20)	5(6)	2.97	.25-.50
Homogeneity						.92	.25-.50
			CROSS 5 (BRF86-03 X DAVIS)				
1	33	18(19)	6(6)	6(6)	3(2)	.46	.90-.95
2	44	25(25)	9(8)	7(8)	3(3)	.28	.95-.99
Pooled	77	43(43)	15(14)	13(14)	6(5)	.46	.90-.95
Homogeneity						.28	.50-.75
			COMBINATION OVER TWO CROSSES				
Pooled	184	105(104)	40(34)	28(34)	11(12)	2.14	.50-.75
Homogeneity						1.29	.25-.50

* expected numbers are in parentheses

CHAPTER V
SUMMARY AND CONCLUSIONS

In this dissertation, results are reported for experiments designed to determine the effects of date of planting and maturity, and harvest time on the occurrence of fungal infection in soybean seeds, to study the genetic basis of resistance to Phomopsis seed decay in PI 417479 and F85-4901, and to study the inheritance of brown radicle, a new phenotype with pigmented pattern in the seedcoat. A series of experiments, planted at six different times, were established in 1986 and repeated in 1987. Five genotypes, F85-994, Forrest, F85-998, F85-1108, and Kirby, and three harvest times, seeds harvested at maturity (H_0), one week later (H_1), and two weeks later (H_2), constituted the treatments. The blotter test was used to evaluate the incidence of fungal infection in seeds harvested at different maturity dates. It was determined that:

1- The highly humid season during seed maturation in 1986 and a relatively drier season in 1987 caused large differences between years in fungal seed infection.

2- Environmental condition during the maturation period affected the incidence of seeds with fungal infection and percentage of seed germination. A period of high moisture is

necessary to achieve extensive infection and low germination.

3- Phomopsis spp., Fusarium spp., and Colletotrichum truncatum were the three most important fungal types found to infect seeds of soybean in Gainesville, FL.

4- A high incidence of seed infection by Phomopsis and Fusarium occurred when maturing seeds where subjected to frequent rainfall regardless of maturity date. Extensive infection by Colletotrichum occurred only in early-planted genotypes with early-maturing seeds subjected to high temperature and heavy rainfall.

5- Date of planting influenced fungal infection of seeds and seed germination in blotter test indirectly because it was partially confounded with maturity date. Early planted early-maturing genotypes presented the highest incidence of seed infection and the lowest percentage of seed germination.

6- Seed infection increased substantially as harvest was delayed after maturity. Seed germination declined proportionally to the incidence of seeds with fungal infection.

7- The most suitable maturity period for effective selection for resistance to fungal infection of seeds seems to be the dates, predicted by the long-term average weather conditions, which have high temperature and high moisture.

The inheritance of resistance to Phomopsis infection of soybean seed was studied in F_2 populations. Two resistant types, PI 417479 and F85-4901, were intercrossed and crossed with susceptible breeding lines and cultivars. The parents and F_2 plants were subjected to either field-weathering conditions (14 days harvest delayed) in 1987, or incubator conditions (4 days at 100% RH; 25 C) in 1988. Based on this study, the following may be implied:

1- Two dominant genes likely control resistance to Phomopsis infection of seeds of PI 417479. Modifying genes are suspected to be involved in the resistance.

2- Results from crosses with F85-4901 were inconclusive.

3- Broad-sense heritability estimates were high, thus effective selection for resistance to Phomopsis infection of seeds can be attained by using appropriate breeding procedures.

4- Percentage of seeds with Phomopsis and with all fungi were highly correlated with percentage of seed germination in blotter tests. Thus, selection based on percentage of seed germination in blotter tests may be an effective method to improve resistance to pathological and physiological causes of seed deterioration.

The inheritance of brown radicle pattern was studied in five F_2 populations from crosses carried out between brown radicle genotypes and cultivars with a normal seedcoat. It was found that:

1- Brown radicle was controlled by a single recessive gene tentatively designated **rd**. The expression of brown radicle may be influenced by modifying genes.

2- A close linkage between the light hilum **I** and **rd** loci was indicated. Progeny tests of F_2 plants will be conducted to elucidate further the linkage relationship between these two loci.

3- Complete independence was found between pubescence color **T** and **rd** loci.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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